

(19)



Europäisches Patentamt

European Patent Office

Office européen des brevets



(11)

**EP 0 705 842 A2**

(12)

**EUROPEAN PATENT APPLICATION**

(43) Date of publication:

10.04.1996 Bulletin 1996/15

(51) Int. Cl.<sup>6</sup>: **C07K 14/00**, C12Q 1/68

(21) Application number: **95115510.0**

(22) Date of filing: **02.10.1995**

(84) Designated Contracting States:

**AT BE CH DE DK ES FR GB GR IE IT LI LU NL PT SE**

(30) Priority: **06.10.1994 EP 94115751**

(71) Applicant: **HOECHST AKTIENGESELLSCHAFT**  
**D-65929 Frankfurt am Main (DE)**

(72) Inventors:

- **Bartnik, Eckart, Dr.**  
**D-65205 Wiesbaden (DE)**
- **Margerie, Daniel, Dipl Biol.**  
**D-60320 Frankfurt (DE)**

(54) **Regulated genes by stimulation of chondrocytes with 1L-1beta**

(57) The present invention refers to the novel use of osteopontin, calnexin and TSG-6 gene product in the diagnosis, prophylaxis or therapy of IL-1 $\beta$  mediated diseases of connective tissues and to novel genes induced or repressed by stimulation of chondrocytes with IL-1 $\beta$  and their use in the diagnosis, prophylaxis or therapy of IL-1 $\beta$  mediated diseases of connective tissues.

**EP 0 705 842 A2**

## Description

The present invention refers to the novel use of osteopontin and calnexin in the diagnosis, prophylaxis or therapy of IL-1 $\beta$  mediated diseases of connective tissues and to novel genes induced or repressed by stimulation of chondrocytes with IL-1 $\beta$  and their use in the diagnosis, prophylaxis or therapy of IL-1 $\beta$  mediated diseases of connective tissues.

Among the diverse biological effect of interleukin-1 (IL-1), are its actions on the metabolism of many connective tissue cell types including articular chondrocytes. IL-1 inhibits proteoglycan (PG) synthesis by chondrocytes and stimulates production of prostaglandin E<sub>2</sub> and metallo-proteinases capable of degrading matrix macromolecules. From experimental results, and from findings of IL-1, PG fragments and proteolytic enzymes in inflamed joints, it was deduced that IL-1 plays a role in cartilage degradation in osteoarthritis and rheumatoid arthritis (Benton HP & Tyler JA. 1988, Biochem. Biophys. Res. Comm. 154, 421-428; Aydelotte MB et al. Conn. Tiss. Res. 28, 143-159; Wood DD et al., Arthritis Rheum. 26, 975-983; Lohmander LS et al., Trans Orthop. Res. Soc. 17, 273). Matrix metalloproteinases are potential candidates for drug interaction at the enzyme level, but relevant molecular targets interfering with earlier processes leading to cartilage degradation are still lacking. Therefore, one objective of the present invention was to identify potential targets for drug modification of IL-1 $\beta$  induced cartilage degradation on the RNA level of human articular chondrocytes from osteoarthritic cartilage.

As an initial attempt to investigate differentially expressed genes in diseased cartilage, total RNA from IL-1 $\beta$  stimulated and unstimulated human chondrocytes was subjected to differential display of mRNA by reverse transcription and polymerase chain reaction (DDRT-PCR). This method can be used to identify and isolate those genes that are differentially expressed in two cell populations (Liang P & Pardee AB 1992, Science 257, 967-971; Liang P et al., AB 1993, Nucl. Acids Res. 21, 3269-3275; Bauer D et al. 1993, Nucl. Acids Res. 21, 4272-4280). The key element is to use a set of oligonucleotide primers, one hybridizing to the polyadenylated tail of mRNAs, the other being arbitrary decamers that anneal at different positions relative to the first primer. mRNA subpopulations defined by these primer pairs are amplified after reverse transcription and resolved on DNA sequencing gels. Band patterns are created, which are characteristic for each RNA population extracted from the cell population under study. For example, 100 different primer combinations should generate a total of approximately 10,000 PCR products for each population, which should represent about the half of all expressed cellular genes. A comparison of the band pattern obtained from two cell populations reveals differentially displayed bands which correspond to differentially expressed genes. Subsequently, differentially displayed bands can be extracted from the gel, reamplified, subcloned and sequenced.

Due to its extreme sensitivity, the appearance of artifactual bands is an inherent problem of the DDRT-PCR method used according to the present application. An additional problem is also the evaluation of complex gene expression patterns. Yet another problem of the present invention is that only minute amounts of RNA are available.

Therefore, it was particularly surprising that the DNA TAU1/1 with the sequences

## TAU1/1(1)

ACATCACCTC	ACACATGGAA	AGCGAGGAGT	TGAATGGTGC	ATACAAGGCC	ATCCCCGTTT	60
CCCAGGACCT	GAACCCGCCT	TCTGATTGGG	ACAGCCGTGG	GAAGGACAGT	TATGAAACGA	120
GTCAGCTGGA	TGACCAGAGT	GCTGAAACCC	ACAGCCACAA	GCAGTCCAGA	TTATATAAGC	180
GGAAA						185

and

## TAU1/1(2)

CTAAATGCAA	AGTGAGAAAT	TGTATTTTTT	CTCCTTTTAA	TTGACCTCAG	AAGATGCACT	60
ATCTAATTCA	TGAGAAATAC	GAAATTTCAG	GTGTTTATCT	TCTTCCTTAC	TTTTGGGG	118

and the DNA TTU2/2 with the sequence

```

5  AACCAGTATT TCAAACTAT TATCTGGATT CAAGATTAGT GTGTAAAGAT TGTTCCTTA      60
    TCAGTAAAAT AGGTCTTCAG ATCTGCATCT GGCCTCTTAG CATGTTTTTC TTCATAGATA      120
    CCGGTTTTGG GGTTCCTTGG TCGGAAGATG AATGGCATT ATAGTCCTCT CCACATTTAT      180
    CTG                                          183

```

10 are 100 % identical to human osteopontin cDNA and 97.2 identical to human calnexin, respectively. This demonstrates that the experimental approach of the present invention worked efficiently, i.e. the use of 100 different primer combinations (25 oligodecamer primers, 4T<sub>12</sub>MN-primers) generated a total of approximately 10.000 PCR products for each population which represent 53 % of all expressed cellular genes. 123 PCR bands out of 10.000 appeared as differentially expressed bands. 53 of the original 123 PCR bands were reproducibly displayed by comparing the PCR band patterns from two patients; of those 68 % arose from IL-1 $\beta$  stimulated chondrocytes.

15 It was further found that osteopontin which is a secreted highly acidic phosphoprotein of 32 kd (Denhardt and Guo (1993) FASEB J. 7, 1475-1482) is surprisingly downregulated in IL-1 $\beta$  stimulated human chondrocytes. This means that osteopontin is involved in IL-1 $\beta$  related diseases of connective tissues, in particular osteoarthritis.

20 Osteoarthritis is characterized as a slowly progressing matrix degeneration with continuing degradation of collagens and proteoglycans and subsequent release of matrix fragments into the synovial fluid. Any disturbance of the normal chondrocyte matrix interactions, for example through a loss of osteopontin, could cause an altered signaling through the integrin  $\alpha_5\beta_1$  and thus changed cellular responses leading to early steps of matrix degradation.

Therefore, one embodiment of the present invention is the use of osteopontin itself or parts thereof, antibodies 25 against it or nucleic acids such as DNA or RNA or parts thereof coding for osteopontin or parts thereof in the diagnoses, prophylaxis or therapy of IL-1 $\beta$  related diseases of connective tissues, in particular osteoarthritis. According to the present application the term "parts" means either at least 8, preferably 12, in particular 15 amino acids in case of proteins or 6-100, preferably 10-40, in particular 12-25 nucleic acids in case of DNA or RNA as hybridization probes. The methods of diagnosing such diseases will be described infra. In addition, quantification on the protein level is possible with osteopontin specific antibodies on Western blots, in immunochemistry, FACS analysis or ELISA based assay systems. The 30 present invention refers also to a diagnosis aid or a pharmaceutical for such use. Osteopontin can be produced for example recombinantly through expression in procaryotes, in insect cells in mammalian cells or in mammalian cells using Vaccinia as detailed in Ausubel et al. 1994 [Current protocols in molecular biology, Chapter 16, John Wiley & Sons, Inc]. The cDNA of Osteopontin is e.g. disclosed in Young et al. (1990), Genomics 7, 491 - 502.

35 Antibodies against osteopontin can be generally produced for example by the method of Neil GA & Urnovitz HB (Trends in Biotechnology, 6, 209-213, 1988) or Köhler G & Milstein C (Nature, 256, 52-53, 1975).

Also calnexin which is an integral membrane protein of 88 kd (Bergeron et al. (1994) TIBS 19, 124-128) is surprisingly downregulated in IL-1 $\beta$  stimulated human chondrocytes compared to unstimulated chondrocytes. This means also that calnexin is involved in IL-1 $\beta$  related diseases of connective tissues, in particular osteoarthritis. In addition, a downregulation of the calnexin synthesis would cause a reduced amount of correctly and completely folded proteoglycans because calnexin is a new type of molecular chaperone that associates with incompletely folded proteins such as proteoglycans. Proteoglycans are highly glycosylated glycoproteins which are of central importance for the maintenance of the cartilage tissue integrity.

40 Hence, an additional embodiment of the present invention is the use of calnexin itself, or parts thereof antibodies against it or nucleic acids such as DNA or RNA or fragments thereof coding for calnexin or parts thereof in the diagnosis, prophylaxis or therapy of IL-1 $\beta$  related diseases of connective tissues, in particular osteoarthritis. The methods of diagnosing such diseases are already described above. The present invention refers also to a diagnosis aid or a pharmaceutical for such use.

Calnexin can be produced for example recombinantly as described above for osteopontin. The cDNA of Calnexin 50 is e.g. disclosed in Galvin et al. (1992), Proc. Natl. Acad. Sci. USA 89, 8452 - 8456. The production of said antibodies are also generally described above.

#### Potential role of identified cDNA fragments in IL-1 mediated cellular processes TSG-6

55 A homology search in the GenBank and EMBL databases revealed a 99.5 % sequence identity of fragment TAU7/2(c) with the gene coding for human TSG-6. TSG-6 (TNF stimulated gene 6) was originally isolated by differential cDNA library screening as a TNF induced gene sequence from human fibroblasts (Lee et al., 1990). It was further characterized by Lee et al (1992) as a TNF and IL-1 inducible, secretory, 39 kDa glycoprotein with extensive sequence homology with a region implicated in hyaluronate binding, present in cartilage link protein, proteoglycan core proteins,

and the adhesion receptor CD44. With the ability to bind HA and with the most extensive sequence homology to CD44, TSG-6 belongs to the hyaladherin family. Wisniewski et al. (1993) detected high levels of TSG-6 protein in synovial fluids of patients with various forms of arthritis. Six normal control patients did not contain detectable TSG-6 protein in their joint fluid, whereas joint fluids from nine rheumatoid arthritis patients contained high, moderate or low levels of TSG-6. Two patients with osteoarthritis had high levels of TSG-6 in their joint fluids. The apparent local source of TSG-6 in the joints are synoviocytes and chondrocytes (Wisniewski et al., 1993). Lee et al. (1992) speculated that TSG-6 could act as a competitive inhibitor of the interaction between CD44 and its ligand(s) and thus might influence the structural organization of the extracellular matrix of connective tissue, resulting in a destabilization of the proteoglycan aggregates.

Hence, an additional embodiment of the present invention is the use of TSG-6 gene product itself, or parts thereof antibodies against it or nucleic acids such as DNA or RNA or fragments thereof coding for TSG-6 gene product or parts thereof in the diagnosis, prophylaxis or therapy of IL-1 $\beta$  related diseases of connective tissues, in particular osteoarthritis. The methods of diagnosing such diseases are already described above. The present invention refers also to a diagnosis aid or a pharmaceutical for such use.

#### Fibronectin

A homology search in the GenBank and EMBL databases revealed a 100 % sequence identity of fragment TFO20/1(c) with the gene coding for human fibronectin.

Fibronectin is a 450 kd glycoprotein with various functions. It acts as an adhesive ligand, as growth or differentiation factor and has chemotactic properties. It is found in the extracellular matrix of most types of cells (Hynes R 1993. *Fibronectins*, In: Guidebook to the extracellular matrix and adhesion proteins. Editors: Kreis T, Vale R. Oxford University Press. 56-58). An enhanced accumulation of fibronectin and fragments derived from it are found in the synovial fluid and on the inflamed synovial and pannus surfaces in the knee joint of patients with rheumatoid arthritis (Dutu A, Vlaicu-Rus V, Bolosiu HD, Parasca I, Cristea A. 1986. *Fibronectin in plasma and synovial fluid of patients with rheumatic diseases*. Med. Interne 24, 61-68). Patients with osteoarthritis, as well, have greatly increased levels of fibronectin in their synovial fluid and on cartilage surfaces (Xie D-L, Meyers R, Homandberg GA. 1992. *Fibronectin fragments in osteoarthritic synovial fluid*. J. Rheumatology 19, 1448-1452). The intraarticular injection of fibronectin fragments causes a severe depletion of cartilage proteoglycans in vivo (Homandberg GA, Meyers R, Williams JM. 1993. *Intraarticular injection of fibronectin fragments causes severe depletion of cartilage proteoglycans in vivo*. J. of Rheumatology 20, 1378-1382), which is explained by the induced release of several proteinases, including stromelysin (Xie D-L, Hui F, Meyers R, Homandberg GA. 1994. *Cartilage chondrolysis by fibronectin fragments is associated with release of several proteinases: Stromelysin plays a major role in chondrolysis*. Arch. Biochem. and Biophysics 311, 205-212). At high concentrations, fibronectin fragments enhance cartilage catabolism through release of cytokines, including IL-1 (Homandberg et al., personal communication).

In respect to these published data, the upregulation of fibronectin by IL-1 can be regarded as a positive feedback regulation, enhancing the self destructive potential of chondrocytes and synoviocytes. With this, fibronectin expression is a direct pharmacological target.

In addition, the sequencing of differentially displayed PCR products discovered also unknown DNA fragments which correspond to differentially expressed genes with or without stimulation of chondrocytes with IL-1 $\beta$ .

# EP 0 705 842 A2

Therefore, another embodiment of the present invention is a DNA containing a DNA selected from the group consisting of

5 TA08/2(2)  
1 CCAAGTTTTT CCAGCAACCC CAAGGGAATA CAGGGAGATC AATGCACCA  
51 AAATGGGAAA AGAAAAATAC TTCGATGCAA TGAAACAAAG CCTTTTCCG  
101 TTCAGTTTCC ATAATTCAGT GGTCAGTTT AAGGCTGCCA CTTGGG

10 TA016/1(2)  
1 GACACGAACA CCACATATTT TTATTGGAGG CCCCATGGCT CCTTGGAAGC  
51 CATTTTGGAA CCAAGGGGAC CCACCTTTTT

15 TA016/2(2)  
1 CTAAATATAT TCTCTAACAA GTTAATCTCT TTCAAATCTA TAGATAAAAC  
51 TAAAAGGATA AGGAACCAAG GTTTAACCGA CCTAGCCAAT TATGGCAATC  
20 101 AACTTGCTT TTTAG

25

30

35

40

45

50

55

EP 0 705 842 A2

TA017(C)

5  
1 CATGAAATAT TTCTTGAGGT AATAAGCTTT TACCAAGCTT ATATTTTGG  
51 GCAATTCAGT TACAATGAGA AAAAAACACA CCAAAGACC AAAAATTTA  
  
101 AAACTCACT TTTCTTGCAA TCATAGACAT TTGCATTATT ATAGAACATT  
151 CAAACAAGTT AGGTGGATAA TTATTGTCTA TAGATAAATA CGATGCAATT  
10  
201 TTTTAAATGT ATGACCGATA CTCCGTATAT ACTTAGATAA CTTATCCAGA  
301 AACCTCAACT GTATTGAACA TTGCTGAGAG AAATCAACAA TAATTTTAAC  
351 AGATATGATG ACAGNAAAA TTGATTGCAT ATCTTTTGC ACTAAACTT  
401 TTATATTAT TT

TA019(C)

15  
1 AGAGCAGGGG TATTTNCGG TTCATACCGC CATGGCTTAA GAAGCAAAAG  
51 TCAATACCT TAGTAGTGGC AAAGATNGAG GAGATAAAAA AGAGCCTACC  
20  
101 CAAGCTGTTG TTGAAGAACA GGTCTTAGAT AAAGAGGAAC CCTTCCAGAA  
151 GNACAGAGAC AGGCTAAGGG TGATGCTGAG GAAATGGCTC AGAAGAAACA  
201 AGAGATTAA

TAU 1/1(2)

25  
1 CTAAATGCAA AGTGAGAAAT TGTATTTTT CTCCTTTTAA TTGACCTCAG  
51 AAGATGCACT ATCTAATTCA TGAGAAATAC GAAATTCAG GTGTTTATCT  
101 TCTTCCTTAC TTTTGGGG

TAU 1/1(1)

30  
1 ACATCACCTC ACACATGGAA AGCGAGGAGT TGAATGGTGC ATACAAGGCC  
51 ATCCCGGTTT CCCAGGACCT GAACCCGCCT TCTGATTGGG ACAGCCGTGG  
101 GAAGGACAGT TATGAAACGA GTCAGCTGGA TGACCAGAGT GCTGAAACCC  
35  
151 ACAGCCACAA GCAGTCCAGA TTATATAAGC GGAAA

TAU1/2(2)

40  
1 CCGGAATGGG GAGCAAACCTA TAAGAACCGG GACCAAGTTT CTCTCTTGT  
51 GCCCTAGTTC CCCCTCCTTT GTATACACCC TCCATCCTGA ATAGACTCTG  
101 GTTCTCAGCG TAACACCGAC AACATTCAAT CCTGTAGAGA AACAAATGTT  
151 AGCTCAGAAG GACACAGCCT TTGAATCATC AGAGAGTT

TAU 7/1(2)

45  
1 GTTAAGAATA ACTAAATAAA AGTTTAAATT AATTTAGGAA TATAAAAAAC  
51 TATTACATT TAATTTTATA ACTGTATCTG CCAAGCAACT TTAATATAA  
101 TTTATTTACC

TAU 7/1(1)

50  
1 CACGCAATGT GAAATAGGCA CATAGGAAGA ATGGGGAAAC CATCCCCTCA  
51 AGCATTTATC CTTTGAGTTA CAAGCAATCC AATTACACTC TTTTAGTTAT  
101 TTTTAAATGT ACAGTTAGGT TATTA

55

EP 0 705 842 A2

TAU 7/2(C)

5  
1 CCTTGAAGAT GACCCAGGTT NCTTGGCTGA TTATGTTGAA ATATAGACA  
51 GTTACGATGA TGTCCATGGC TTTGTGGGAA GATACTGTGG AGATGAGCTT  
101 CCAGATGACA TCATCAGTAC AGGAAATGTC ATGACCTTGA AGTTTCTAAG  
151 TGATGCTTCA GTGACAGCTG GAGGTTTCCA AATCAAATAT GTTGCAATGG  
201 AT

TAU10(1)

1 GGAGATGACA TTTGCTTTGG GCAGAGGCAG CTAGCCAGGA CACATTTCCA  
51 CTATAATTTT ACAAAGTTAA ATTTATAAGC TAGCATTAAAG TAAAGTGAAG  
15  
101 TTCCAGCTCC CTTGCTAAAA ATAAC TAGAG GTAATAATTG GTATTCAGCT  
151 AACTCATTTA CATCATAATG TGTGTGAAA A

TAU12/1(2)

20  
1 TATAAAATAT AAATTATATT ATAAATCATG TATTATTTAT AAAATTATAT  
51 TATAAATTTA TAAAAATATA AATTATATTT TAGGCTTAAT GTATAACGAA  
101 TATAAATTAT TAATAAGCAT ATGA

TAU 12/1(1)

25  
1 TGAATTAAC TGTNCTTGTA GGTCTGTCTT TTATACATGT GTGAGTTTTT  
51 CTTTACAATA GATTCCTAGC ATTGGGATTG CTAGGTCAGA TGGTATGCAC  
101 ATTTGACATT TTGATTGATA GCACCAGATT GCTTTGTTAA AAAATTTTNN  
30  
151 TTTATAGTTT ACATTATCTT TGTACAATAG ATGTTCTCTT TCGAC

TAU 12/2(1)

1 GGGAAGTGAA TTGAAAATAC TTCTTTNTCA ACATAATTTT NGGGTTTTGA  
51 AATTGTGTTT GGGTTTTTCAG GAAATGGGTG GTAATCTTGT ATTAGACTGAA  
35  
101 AAAAAAGTGAA TTTTAAAATT CTCAGTGAAG AAGCAAATGA TTTATTTTTC  
151 ATAGA

TAU12/3(2)

40  
1 TGTTCGTGTA ACTGTTCTAA TTGTGTCTTT GTTACTTCCA GTGCAACCCT  
51 TTCAGGTAAG

TAU12/3(1)

45  
1 CTAAAGAACT TGGTATCTCT ATTAAAGCAC ACGAACCTCC AAGGAAAATA  
51 GAGCGATTTA CTCTTCTCAT ATCAGTGCAT ATTTATAAGA AGCACGGAGT  
101 CA

TAU13/1(1)

50  
1 AGTCATCAAT TCCTTTTTAT CTGTAATTAC ACATTTGTTT TTATTTCAAA  
51 GTAATTATAA GGTGTTATAT TGCATATAAT CAGAAAAC TAATGGAATA  
101 AAATTTTAGT AAGCCCGGCC CCTTTGACCG ATACAGAAAA CTTGA

EP 0 705 842 A2

TAU 13/3(2)

5  
1 TATATGGCAG TCTAAAGCAT CAAAGATTG CATCAACATC TTTCATTTTA  
51 GACATCTCCT TGCAATGTAA AATATCATGT ATCAACAACA TCTGGTGCAA  
101 ATCCATGAGT CTAACCTGAC ATTATCTTA GCTCGATTAT TATTCCTTCG  
151 TACAGTCGAT GTAAACAATA CAGAAAGAGG ATTATTAAGA ACAGTTT

TAU 13/3(1)

10  
1 ATTCATGAAA TGGTCTATAT GCATGATATT GTAAATTTCGG ACTCCAAACC  
51 GAAACCAAGG ATTCCGTTAC AAAAATTCCT TAATGCTGAG AATGTTCTCA  
101 CGCAACAAC ATCATGGACA TTAAATTCAA GATATGTGAA TGTAAATTCT  
151 GTCAATAAAG TCAACGTAAA GAGTAAAGTT AAAAACAGTT ATATCTNNNC  
15 TGTCAATGAT GAGTTTAGTT TAACAGATGA TGAATCAATT CT

TCO 16/1(C)

20  
1 CAAAGTGTTC TTGGTTTTGA GAGAGAGAGA GATTGAGAGA CAGAGAGAGA  
51 GAGAGAAACC AAGGGATCAT GATAGTTATA GTCAAATACG AGGTTGGATT  
101 ATCTTTTGAA AATGTGTTGG TTCTGTGATA CAAGAGGAAG CTAAGACATA  
151 TCGTGGAAAC ATCTCCCCC TCCACCTTAA TATCAAGAAC AAATTGTGGA  
201 ATCTAATGTT AATGAGAAGT AGTCCCCAC TGTGTCAGAT G

TCO16/2(C)

25  
1 NCATCTGACA CAGTGGGGAA CTAATTCTCA TTAACATTAG ATTCCACAAT  
51 TTNNNCTTGA TATTAAGGNN NNNNNGGGAG ATCGTTTCAC GATATCGTCT  
30 101 TAGCTTCCTC TTGTATCACA GAACCAACAC ATTTCAAAG ATAATCCTTC  
151 CTCNNTTGA CTATAACTAT CATGATCCCT TGGTTCTCTC TCTCTCTCTG  
201 CTCTCTCATC TCTCTCTCTC TNA AACNAA

TCO17(C)

35  
1 ACAGTAGTTA GGAGTTTCTT TACTTACAAA ATCACTGGAA ATGATTAAAT  
51 TGCTTTTCCC CCTCCCCAGA GGTGCATTTT TCTTATTTC ATATAGTAAA  
101 GTTGAGCTTT TACAGTGCAT AATGTGACAT TTGGAATGCT TATCAACTGC  
151 ATGTAAACAT TAATAACCT

TCO18(C)

40  
1 GTAAATGGTA TTANNNGCTG AAGAAAAAAA ATTTTCAAG ACCTCTGTTT  
51 TTAACTGAA CTTTATCATT GGCATTGTGG GCTTTGAAGT TGCTGGGATA  
45 101 AATTAATATA ATTAATAAAA AGACTGAATT TAATTGCAA AAAAAAAAAA  
151 AACAATAAGT GTGGTGAT

TCU2/1(1)

50  
1 AAGAAATTAT CCAGTTATTT ACAAGGCCAC TGATATTTTA AACGTCCAAA  
51 AGTTTGTTTA AATGGGCTGT TACCGCTGAG AATGATGAGG ATGAGATGA  
101 TGGTTGAAGG TTACATTTTA GGAAATGAAG AAAGTTAGAA AATTAATATA  
151 AAGACAGTGA TAAATACAAA GAAGATT

55



EP 0 705 842 A2

TCU2/2(1)

5  
1 CGGGTTAATA TTATCCTCTA GTATAAGTGA ATTACTAGTT TCTCTTTATT  
51 TAGACAAACA CACACACACC AGATAATATA AACTTAATAA ATTATCTGTT  
101 AATGTAGATT TTATTTAAAA AACTATATTT GAACATTGGT CTTTCTTGGA  
151 C

TCU9/1(2)

10  
1 ACATAACAGC TTTTATACAA TGATAAGGAC ATATCATTG TTTACAAAGA  
51 AAGTCTAAAA TTTCAAGAAC ATTCAAAGAG CTAACACAGT AAAGGTCATG  
15  
101 CAAGTTCTAG AATAGTGAAT CATGACAGAA CTCATTCAAT TTATCCTTTA  
151 TCTCC

TCU9/2(2)

20  
1 AAGTATGGGT AGCTAAATTT GCATTAAATT AAAAGTACAT ATAATGCAAC  
51 ACCACTCTAC ATCTGTATAC CTACGAATGT ATGTGTACTA CACACCCTTA  
101 AAATGTTTTT CAAAGTCTTA ATATATTAGA ACATGTTTTC ATTTTTCAT  
151 GGGATGTTAA TACTATTCTA TGATTAAGAA AATACTAG

TCU10(2)

25  
1 AATACAGTTA TTCTAGCTTT TCATATTCAA TTTGAATGAT CAGAAAAGTA  
51 TATTAGTCAC ACAGAATTAA ATATTTTAGA TAGTAAGAAT C

TCU14(2)

30  
1 GAAGTGAAAG TCAGCCCTTT AGCTATTATT TATTGCTTTA TTAGAGCAGA  
51 GGGAAAGTGAC ACTCATTGCC TTCACAGAGC TCTGCAGAAA TATATGCACA  
101 GAGTGGTCAA TGCCAACATC TGAGTAAGTC TTCCAAA

TGO20(2)

35  
1 CAGAACATTA GGATTTATTC CTTGATTAGT TCAAATGATT TCAACAGCTG  
51 AATTCCTTGA GATGTGTAAG GCAGGTTGGT CCTTTGGATG GACTGTAGAC  
101 TGAAACTTCC TATAACTGTA GTGATATGTA CACAGCTACA TAGCAAAGTG  
40  
151 CTTCAATTATG AAAATGAAGA A

TGO20(1)

45  
1 CAGTGTGAGA GTCTCATTTT TATGCACAGT GTTTCTCAGG AGGATGGAGC  
51 TAGTTAGCTG TCTGTTGTCT GTAGCCCAGC TTGATAATGG AACTATACAG  
101 CGAAGAGACA ATCTCTGGCA AGTTTTTGTA GAA

TGU5(C)

50  
1 TTAGAGTAAA ATTCCAAATA AATGCTTTGC TCCAAAATTA CACTAACCAG  
51 GCTGGGTCTC TATCATACAT CTTCAATACC CTCAAACCTA GATTGTAAAG  
101 TGAAAAAGT GATTAGCNNT TCCATTGTGT CATTCTGTCA CTCACATTCT  
151 TAGGCATTTT AAGGATGAGC AACCTTTGTT TCAGAAAGGG TAAGTAATTA  
201 GCCCCCTGGA GGTACATAG TTATAATTTA GTCTTCAGAA TCCGTTCGAA

55

EP 0 705 842 A2

251 GGGNNNNGTT ACTATTTTTA AGATAATTAG AACCCACCTT GTAGCAATAA  
301 AAGTTTTCTT GTCTTTG

5

TGU8(2)  
1 GCGAAAGACT AATCGAACCA TCTAGTAGCT GGTTCCTCC GAAGTTTCCC  
51 TCAGGATA

10

TGU9/1(2)  
1 TTAATGTTTA AATACTACTT TTTTTTCAAG CTTGCCCTAG ATACCAACTG  
51 TTTATCTAAC ACACAATTCC AGTGTTGCCA AGCCTCATGC CAATTTGAAG  
101 GGAACAGCCA AAACCTATGC ATTCATATAA AAAGAGTCTC TAGGCTCTTA  
151 TATCTACATT ATAATTTT

15

TGU9/2(2)  
1 GGAATAACAT TTTTTTATGA GGAACCCCTT TAAATGGAT GCACACAGTG  
20 51 GCATTTTCTC CTAGGCTCAA AGCTGAGTAC ACTCCCGTAA TTTTAATAAT  
101 ATTTTAGGCA AGTCCTATGA CAATTATACC AACAAGTTTC TTCAACCCCA  
151 CCACCACCCC ACCATCTCTA TGC

25

TGU12(C)  
1 GGAGGAAGCT TTATTTGGGA AGAGTGC GGT TCNNTCGGCC CTGATCAGCT  
51 CTAGCCTGCC CACCCCATCT CAGCCAGGCG GCTTTACTTC TTCCTGAGCT  
101 TCAGGTCTTT CTTCTCCTG ATTTCTTGG CCAGCTCCCC AATCAATCTC  
151 CAGTACTCAT TGAACCTGAG CTCCGAGNCC TGATTCACAT CCAAGCTCTT  
30 201 CATCTTCT

35

TGU13/1(C)  
1 GGATGTGGTA GTTGATCTTT AATGCCCAT CTAGGTCGGA AAAATCCATG  
51 ATCCTAACTT TTAAGAGAAG GTTGGTAACT CTACTTAGGA CTTTTTTTGG  
101 TAAGAGGAAT AATGTAGCCT CACCCTTATC TTTCTGAAA TGTTTAAACC  
151 ACTGAAATAT GGAGATCAA TCCAGCTTAC ACACTGGTAA CTCAAATACT  
201 ATTTTTTTTT TAACTATCT TTTCTAACT AATCACCCCT CTGTACATA  
251 GAACTTTCTA TCTCAGTGCC AATTCTTAGA GGTGATGCA AACAGCTCTC  
40 301 CAGAGAGCCT GTGCTATTGT TC

45

TGU13/2(2)  
1 GGGGTGTACA TTTTATTGGA AACCTTAAAT ACTGTTTACA AAGAATATAT  
51 CTTCAATCAA GGTCTTGCCG AGCCTACACA GAAAATGAA GCTTTTTGGG  
101 TTAGGGGCAA GGATATATAC AGTACAGAGG ACAAAGA

50

TT016/2(C)  
1 ACATTCATTA AAGATGAACT TTCAGCATCT TCACTTGAAG ATCCATCAGA  
51 TGATTCTGAG AGGCAGGTCT CCCCCAAAA TCCACCGCAT GTATTCTTTC  
101 GTTTAGAATC TGAAAGCCTC TTTCTTTTCA GGCTTGATGA CTCTTCTAAG  
151 GTATTTGTTA TGCCTCTCTT CTGGGTTTTT CGTTTTGCCT TATCAAGTAG

55

EP 0 705 842 A2

201 CTNAAATTCA AACACCATGG CAANAGAAAC TGCTTCTAT

5 TTO20/1(C)

1 CCACCAGCCT ACTGATCAGC TGGGATGCTC CTGCTGTCAC AGTGAGATAT

51 TACAGGATCA CTTACGGAGA AACAGGAGGA AATAGCCCTG TCCAGGAGTT

101 CACTGTGCCT GGGAGCAAGT CTACAGCTAC CATCAGCGGC CTTAAACCTG

10 151 GAGTTGATTA TACCATCACT GTGTATGCTG TCACTGGCCG TGGAGACAGC

201 CCCGCAAGCA GCAAGCCAAT TTCCATTAAT TACCGAACAG AAATTGACAA

251 ACCATCCCAG ATGCAAGTGA CCGATGTTCA AGACAACTGT TTTAATAAAA

15 301 GATTACATT CCAC

TTO20/2(2)

1 TTGGTACCAC AGTCACAGAA CTGGGGGTCA TTTTCTAGAT GAAACAAACG

51 GAACAAGTTC TCTTCCAACA AAGAAATGTA CTGTAGAAAT TAATTTCCTC

20 101 CATGAATTTT ATATATTGTG TACAAATATA AGGTATGTAT CTGAATACAA

151 AG

TTU2/1(2)

25 1 CTAGAACTTC CAAAGGCTGC TTGTCATAGA AGCCATTGCA TCTATAAAGC

51 AACGGCTCCT GTTAAATGGT ATCTCCTTTC TGAGGCTCCT ACTAAAGTCT

101 ATTTGTTACC TAAACCTTAT GTGCCTTAAC AGGCCAATGC TTCTCG

TTU 2/2(C)

30 1 AACCAGTATT TCAAACTAT TATCTGGATT CAAGATTAGT GTGTAAAGAT

51 TGTTCCTTA TCAGTAAAT AGGTCTTCAG ATCTGCATCT GGCCTCTTAG

101 CATGTTTTTC TTCATAGATA CCCGTTTTGG GGTTTTTGCG TCGGAAGATG

151 AAGTGCAGTT TATAGTCCTC TCCACATTTA TCTG

35 TTU3(1)

1 GGGTAGAAAG CTGAATAATT TATGAAGGAG AGGGGTCAGG GTTGATTCCG

51 GAGGACCTAT TGGTGCGGGG GCTTTGTATG ATTATGGGCG TTGATTAGTA

40 101 GTAGTTACTG GTTGAACATT GTTTGTGGT GTATATATTG TAATTGAGAT

151 TGCTCGGGGG AATAGGTTAT GTGATTAGGA GTAGGGTTAG GATGAGTGGG

201 AAG

TTU 5/1(2)

45 1 GACAAAAAAA AAAAAACAGG TTTTAAAGCT AGAAATGAAA AGCTACTTAA

51 GTATCTTAAA GGATAAGTTA CTTTATTATA CACTAGAAAC ATACACAATA

101 GCTGAAAACT TAAAAATCT CACACTGCTG AATGTCTCTG CTGGCTG

50 TTU5/2(2)

1 GCATCCATTG TACATTGTTT GGTTCGAGGT TACCATGAGG CCTGTAAATA

51 CTATCTTATA ATTATTATT TCAACCTGAT AAAACTTAAC ACTATTGCA

101 TAAACAAACA AACGAAAA

55

## TTU9/1(1)

1 TAAATACTG GTTCTTTTAT TCTGCAATAT TTTTAAAAAT CACATTTTCA  
 51 GCCAGGCGCA GTTTCCACACA CCTGTAATCC GGCACTTTGG GAGGCTGAGA  
 101 TGGGTGGATC ACAAGGTAGG AGATCAAACA TCCTGGCCAA CATGGTGAAC  
 151 CTGTTTACT

## TTU9/2(2)

1 CAAGTATGGG TAGCTAAATT TGCATTAAAT TAAAAGTACA TATAATGCAA  
 51 CACCACTCTA CATCTGTATA CCTACGAATG TATGTGTACT ACACACCCTT  
 101 AAATGTTTCA AAGCTTAATA TATTAGAACA TGTTTTCATT TTCAGGGAG

## TTU13(2)

1 GGAAATACAC TAGCATGTGA GCACTGTATA TAAAGCTTGA GGTTAGGAGG  
 51 TAAATGAAA GAAATCATT TTAACCTCTA AGATGT

## TTU13(1)

1 TGAATTAAAT GGAATCGTTG AAAGGACAAG GAGATCGGTA ATATCTCTCT  
 51 AAAGAACTTA TATACTAAAA TCTGTAATTG CCTGTACCAA AAGTTTTAGT  
 101 CTTCTTTT

or an analog thereof. In accordance with the invention, the term "analog" includes nucleic acids which code for the same protein sequence due to the degeneration of the genetic code, for a protein having conservative amino acids substitutions or deletions that do not eliminate the characteristic feature of this protein, or for a protein having at least about 85 %, and more advantageously at least about 90 %, in particular 95 % amino acid sequence homology.

Other embodiments of the invention provide a vector containing said DNA and a host cell containing said vector.

According to the general knowledge one skilled in the art can also use said nucleic acids of the present invention as a hybridization probe to detect the corresponding genes in an organism or in a sample from an organism or gene mutations thereof.

Therefore, an additional embodiment is a method for isolating a gene which can be induced or repressed by treating chondrocytes that contain this gene by IL-1 $\beta$  containing the steps:

(a) hybridizing a DNA of the present invention under stringent preferably high stringent conditions against DNA or RNA containing said gene, preferably DNA or RNA isolated originally from chondrocytes, in particular human chondrocytes; and

(b) isolating this gene by methods known to a skilled person in the art.

According to the present invention the term "stringent conditions" means hybridization conditions comprising a salt concentration of 4 x SSC (NaCl-citrate buffer) at 62-66°C, and "high stringent conditions" means hybridization conditions comprising a salt concentration of 0,1 x SSC at 68°C. The length of the probes are 6-100, preferably 10-40, in particular 12-25 nucleic acids long.

Yet another embodiment is a process for expressing a gene isolated according to the above-described process containing the steps:

(a) cloning said gene into a suitable expression vector such as the pET series (Studier et al., 1990. Methods in Enzymology 185, 60) for procaryotic expression or the vector CDM8 for mammalian expression (Aruffo and Seed, 1987. Proc. Natl. Acad. Sci. USA 84, 8573) or any other expression system known to one skilled in the art; and

(b) expressing said gene in a suitable host cell such as BL21 series (Studier et al., 1990, supra) for procaryotic expression or COS, cells for mammalian expression (Aruffo and Seed, 1987, supra) or any other expression system known to one skilled in the art;

5 or a method for producing a protein containing the steps:

(a) culturing a suitable host cell, in particular the above mentioned, containing a vector, in particular an expression vector such as the vectors mentioned above which contains a DNA or a gene of the present invention; and

10 (b) isolating the expressed protein for example by ultrafiltration, precipitation with chaotropic agents such as urea or column chromatography on e.g. ion exchange chromatography columns as detailed in Ausubel et al. 1994 (supra).

A further embodiment is a diagnostic aid containing a DNA or parts thereof or a gene or parts thereof of the present invention. In particular, quantification of the genes can be achieved on the RNA level by Northern blotting with gene specific probes of the present invention or with gene specific primers in a PCR reaction. Such primers can be synthetically produced using the DNA sequences of the present invention or the sequences of the corresponding genes. Therefore, said nucleic acids are useful for the diagnosis of IL-1 $\beta$  related diseases of connective tissues, in particular osteoarthritis or rheumatoid arthritis.

20 These nucleic acids can also be used to evaluate the expression of certain genes in small cartilage biopsies and to use these ultimately as disease-specific markers and/or as predictive markers for disease progression of e.g. osteoarthritis. The hybridization conditions can be the same as described above.

Said nucleic acids, however, can also be used for the therapy against the diseases mentioned or for the production of a pharmaceutical.

25 Therefore, another embodiment of the present invention is also the use of said nucleic acids for the production of a pharmaceutical. For example, as described by Uhlmann & Peyman (Chem. Rev. (1990), 90, 543), Milligan et al. (J. Med. Chem. (1993), 36, 1923) or Stein & Cheng (Science (1993), 261, 1004) such nucleic acids can be used as antisense oligonucleotides or triple helix forming oligonucleotides for the inhibition of gene expression. This is in particular useful if such a disease is caused by the overproduction of a gene product which is directly or indirectly regulated by IL-1 $\beta$  in chondrocytes. The nucleic acids can additionally be modified in order to increase e.g. the stability against nucleases as described e.g. in the literatures mentioned above.

30 Finally, also the gene product itself produced by a method of the present invention can be used as a pharmaceutical. In the following the invention is in particular described by the examples and tables:

#### Description of the Tables

35 Table 1 gives an overview on used primers and the complexity of the detected differences in expression.

Table 2 summarizes the result of the sequencing of differentially displayed PCR products after their elution from the sequencing gel, reamplification and subcloning into the pCRII vector. The sequences of TAU1/1(1) and TAU1/1(2) are 100 % identical to human osteopontin cDNA, the sequence of TTU2/2 is 97.2 % identical to human calnexin. bp = base pairs, IL-1 = Interleukin-1 stimulation, Stat. sig. score = statistical significance score: a feature of the BLAST database searching program. This score is determined using an implementation of Karlin's significance formula (Karlin, S. and Altschul, S.F. 1990. Methods for assessing the statistical significance of molecular sequence features by using general scoring schemes. Proc. Natl. Acad. Sci. USA, 87:2264-2268), which calculates the Poisson probability that the observed sequence similarity will occur by chance based on the size and composition of the sequence database as well as on the size and quality of the match. The smaller this number, the more it is likely to see sequence similarities.

#### Examples

##### Cell culture

50 Articular cartilage specimen were obtained from two patients (male 65 years old and female 73 years old) undergoing total joint replacement surgery for osteoarthritis. None of these individuals had received treatment by radiation or chemotherapy. Articular cartilage slices were aseptically dissected from both femoral condyles, tibia plateaus and patellae and subjected to sequential enzymatic digestion with pronase and collagenase as described (Häuselmann HJ et al. 1992, Matrix 12, 116-129) Since it is known that the alginate gel suspension system retains the chondrogenic phenotype [Lohmander LS et al. 1992, Trans. Orthop. Res. Soc. 17, 273.] 4 x 10<sup>6</sup> chondrocytes were suspended in low viscosity alginate (4 x 10<sup>6</sup> cells / ml 1,25 % w/v alginate in an isotonic buffered solution) and expressed through a 22gauche needle into 102 mM CaCl<sub>2</sub> solution to form cell entrapping beads which are 1,5-3 mm in diameter and spherical. Alginate beads containing a total number of 2 x 10<sup>7</sup> cells were fed daily for the first three days with medium F12 / DMEM (50/50)

## EP 0 705 842 A2

and 10 % fetal calf serum (Sigma) with 25 µg / ml ascorbate and 50 µg / ml gentamycin and were then subdivided into two populations for further three culture days in the presence or absence of 5U / ml rh IL-1β (Genzyme). For cell recovery, alginate beads were finally dissolved into dissolution buffer (55 mM sodiumcitrate, 30 mM EDTA, 0,15 M NaCl) and placed at room temperature for 10 min. Viability was checked by eosin-red exclusion and cell number was determined.

5

### Primer syntheses

Arbitrary oligodecamer primers OPA6 to OPA10, OPA16 to OPA20 and degenerate anchored oligo-dT primers (T<sub>12</sub>VN) were synthesized using the 392 DNA synthesizer (Applied Biosystems) and purified by denaturing polyacrylamid gel electrophoresis. Some oligodecamer primers, U1 to U15 were purchased from Biometra (Göttingen, FRG).

10

15

20

25

30

35

40

45

50

55

# EP 0 705 842 A2

List of all degenerate 3' oligo dT-primers [T<sub>12</sub>VN] used for DDRT-PCR:

Primer	Sequence 5' to 3'
T <sub>12</sub> VA	5'-TTTTTTTTTTTTTV A-3'
T <sub>12</sub> VA	5'-TTTTTTTTTTTTTV T-3'
T <sub>12</sub> VA	5'-TATTTTTTTTTTTT VG-3'
T <sub>12</sub> VA	5'-TTTTTTTTTTTTTT VC-3'
V = dA, dG, dC; N = dA, dT, dG, dC	

List of all arbitrary 5' oligodecamer primers used for DDRT-PCR:

Primer	Sequence 5' to 3'
OPA 6	GGTCCCTGAC
OPA 7	GAAACGGGTG
OPA 8	GTGACGGGTG
OPA 9	GCGTAACGCC
OPA 10	GTGATCGCAG
OPA 16	AGCCAGCGAA
OPA 17	GACCGCTTGT
OPA 18	AGGTGACCGT
OPA 19	CAAACGTCGG
OPA 20	GTTGCGATCC
U1	TACAACGAGG
U2	TGGATTGGTC
U3	CTTTCTACCC
U4	TTTTGGCTCC
U5	GGAACCAATC
U6	AAACTCCGTC
U7	TCGATACAGG
U8	TGGTAAAGGG
U9	TCGGTCATAG
U10	GGTACTAAGG
U11	TACCTAAGCG
U12	CTGCTTGATG
U13	GTTTTTCGCAG
U14	GATCAAGTCC
U15	GATCCAGTAC

## RNA isolation and cDNA synthesis

Total RNA from cultured articular chondrocytes was prepared according to a single step method Chomczynski and Sacchi (Chomczynski P & Sacchi N 1987, Anal. Biochem. 162, 156-159) and incubated with 10 U RNasefree DNaseI (Gibco, Eggenstein, FRG) for 30 min at 37°C to remove chromosomal DNA contamination of RNA. After extraction with phenol/chloroform (3:1), the supernatant was ethanol precipitated in the presence of 0.3 M NaOAc and RNA was redissolved in DEPC treated water. 0.4 µg total RNA was then reverse transcribed using 200 U M-MLV (Moloney murine leukemia virus) reverse transcriptase (Gibco, Eggenstein, FRG) in a 40 µl reaction volume containing 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 10 mM DTT, 3 mM MgCl<sub>2</sub>, dNTP mix (dATP, dTTP, dCTP, dGTP) of 200 µM each, 40 U RNase inhibitor (Boehringer Mannheim, FRG) and 2.5 mM degenerate oligo-dT primer (T<sub>12</sub>VN) at 37°C for 1 h. Reactions were terminated by heating for 5 min at 95°C.

## PCR amplification

cDNAs were amplified in a DNA thermal cycler (Perkin Elmer, model 480) in 20 µl PCR reactions containing 2.5 µM of the original T<sub>12</sub>MN-primer used in cDNA synthesis in combination with 0.5 µM arbitrary upstream primer, dNTP mix (dGTP, dCTP, dTTP) of 0.5 µM each, 10 µCi α-[<sup>35</sup>S]dATP (1000 Ci/mmol, 10 mCi/ml), 10 mM Tris-HCl (pH 8.3) 50 mM KCl, 1.5 mM MgCl, 0.001 % gelatin and 2.5 U AmpliTaq DNA polymerase. Light mineral oil was overlaid and thermal cycling was performed as follows: 94°C for 30 seconds, 40°C for 2 min and 72°C for 30 seconds for 40 cycles followed by 5 min postextension at 72°C. AmpliTaq DNA polymerase was purchased from Perkin-Elmer (Weiterstadt, FRG) and α-[<sup>35</sup>S]dATP was obtained from Amersham-Buchler (Braunschweig, FRG). After addition of 5 µl stop buffer (95 % formamide, 20 mM EDTA, 0.05 % bromophenolblue and 0.05 % xylene cyanol) radiolabeled PCR-fragments were then displayed on 6 % acrylamide/7 M Urea high resolution sequencing gels of 35 x 43 cm in size; dried gels were exposed to X-ray film (Kodak X-OMAT) and exposed for 48 h, which allows rapid identification of differentially expressed genes by side by side comparison of DDRT-PCR band patterns.

## Elution, reamplification and cloning of PCR fragments

PCR fragments identified as differentially expressed bands were cut from acrylamide gels, transferred into Eppendorf tubes and rehydrated for 10 min with 100 µl 10 mM Tris-HCl and 1 mM EDTA at room temperature. After boiling the gel slice for 15 min, the PCR fragment was recovered by ethanol precipitation in the presence of 0.3 M NaAc and 20 µg glycogen as a carrier and redissolved in 10 µl sterile water. 5 µl of this volume was used for reamplification by PCR using appropriate primers and conditions described above except for dNTP concentration of 20 µM and no radioisotope. The reamplified PCR product was visualized by electrophoresis on a 2 % agarose gel and eluted from the gel by ultrafiltration using Ultrafree MC-filters (Millipore). Purified PCR fragments were then cloned into the pCRII-vector (Invitrogen, De Schelp, NL) by the TA cloning method (Kovalic D et al. 1991, Nucleic Acids Research 19, 4640), which allows in-vitro transcription and sequencing from the plasmid.

## Sequencing

Plasmid DNA sequencing of subcloned PCR fragments with either SP6(2) or T7(1) primer was carried out using the chain-termination DNA sequencing method, as described by Sanger et al. (Sanger F et al. 1977, Proc. Natl. Acad. Sci. USA 74, 5463-5467.).

## Sequence analysis

The sequence analysis revealed the sequences of cDNA clones TAO8/2(2), TAO16/1(2), TAO16/2(2), TAO17(c), TAO19(c), TAU1/1(2), TAU1/1(1), TAU1/2(2), TAU7/1(2), TAU7/1(1), TAU7/2(c), TAU10(1), TAU12/1(2), TAU12/1(1), TAU12/2(1), TAU12/3(2), TAU12/3(1), TAU13/1(1), TAU13/3(2), TAU13/3(1), TCO16/1(c), TCO16/2(c), TCO17(c), TCO18(c), TCU2/1(1), TCU2/2(1), TCU9/1(2), TCU9/2(2), TCU10(2), TCU14(1), TCU14(2), TGO20(2), TGO20(1), TGU5(c), TGU8(2), TGU9/1(2), TGU9/2(2), TGU12(c), TGU13/1(c), TGU13/2(2), TTO16/2(c), TTO20/1(c), TTO20/2(2), TTU2/1(2), TTU2/2(c), TTU3(1), TTU5/1(2), TTU5/2(2), TTU9/1(1), TTU9/2(2), TTU13(2), TTU13(1) disclosed on pages 7 to 14 of the specification.

Searching for homology between subcloned PCR fragments and sequences already listed in one of the DNA databases (GenBank or EMBL database) was performed using the FASTA program developed by Pearson and Lipman (Pearson W & Lipman DJ 1988, Proc. Natl. Acad. Sci. USA 85, 2444-2448) included in the GCG software package (Genetics Computer Group, Madison, USA).



## Northern-blot analysis

Cell culture and isolation of RNA was performed exactly as described above. 10 µg of total RNA from both IL-1β stimulated or not stimulated chondrocytes were denatured by heating at 65°C for 10 min in a solution of 50 % formamide, 20 mM MOPS and 2.2 M formaldehyde, separated through a 1 % agarose gel containing 2.2 M formaldehyde in 1 X MOPS and transferred to positively charged nylon membrane (Amersham) by standard blotting procedures [Maniatis et. al 1992]. After UV crosslinking, the blots were prehybridized for 1 h in rapid-hyb-buffer (Amersham) at 65°C. A 330 bp cDNA corresponding to nts 61 to 390 of human osteopontin cDNA (GenBank J04765) and a 340 bp cDNA corresponding to nts 881 to 1220 from human calnexin (GenBank M94859) were radiolabeled for hybridization with α-[<sup>32</sup>P]dCTP (3000 Ci/mmol, 10 mCi/ ml) using random nonamer primers (Amersham) up to a specific activity of ~ 1,5 x 10<sup>9</sup> dpm / µg DNA. Hybridization was performed for 2,5 h at 65°C in prehybridization solution with 2 ng / ml of labeled probe added. The blot was subsequently washed in 2 X SSC, 0.1 % SDS at 37°C for 15 min (1 X SSC = 0,15 M NaCl, 0.015 M sodium citrate, pH 7,0), followed by two successive washes with 1 X SSC, 0.1 % SDS at 65°C for 10 min respectively. If necessary, a final high stringency wash was performed with 0.1 X SSC, 0.1 % SDS at 65°C for 15 min. The blots were then analysed by autoradiography using Kodak X-Omat films at -80°C with intensifying screens for 2-7 days and intensity of bands was quantified with a phosphorimager (Biorad, model GS-250). All blots were stripped with boiling 0.5 % SDS solution and reprobed with labeled β-actin to demonstrate equal loading of RNA in each lane.

## Northern hybridisations (Results)

Fragment TAU7/2(c), identical to TSG-6, was differentially upregulated in IL-1 stimulated cells. This is in concordance with Lee et al. (1992) which reported for TSG-6 a TNF-α and IL-1 mediated upregulation. Fragment TAU1/1, identical to human osteopontin and fragment TTU2/2, identical to human calnexin, both were weaker expressed in IL-1 stimulated chondrocytes compared with the unstimulated cells. To validate our differential display data, we performed Northern analyses of Osteopontin and calnexin expression in IL-1 stimulated and unstimulated chondrocytes originating from a third patient. Both messages were again downregulated. A phosphorimager quantification revealed an osteopontin downregulation by 79% and a calnexin downregulation by 40% in the RNA population from chondrocytes of the third

patient.

Table 1: Current results of differential display reverse transcriptase PCR (DDRT-PCR) to reveal differential gene expression by chondrocytes with and without IL-1 $\beta$

Overview on used primers and number of analysed bands

DDRT-PCR primercombination		putative differential expressed genes by side comparison	reproducibility of DDRT-PCR band pattern from first to second, third or fourth DDRT-PCR (same patient <sup>1)</sup> (other patient <sup>2)</sup> )	eluted from gel and reamplified in PCR	cloned into pCRll vector by TA cloning method verified by PCR	PCR-fragment sequenced using SP6 or T7 promoter
3'-Oligo dT-primer (downstreamprimer)	5'-Oligodecamer (upstreamprimer)					
T <sub>12</sub> M*A	OPA 6 - OPA 10	25 out of ~ 4000 bands	7 not done	6	1	1
T <sub>12</sub> M*T	OPA 16 - OPA 20	19 out of ~ 4000 bands	13 9	12	12	12
T <sub>12</sub> M*G	U 1 - U 5	31 out of ~ 4000 bands	not done	11	10	10
T <sub>12</sub> M*C	U 6 - U 10	27 out of ~ 4000 bands	not done	12	11	11
	U 11 - U 15	21 out of ~ 4000 bands	not done	11	10	10
total 4 x	25	total 123	total	total 52	total 44	total 44
= 100 combinations						

\* means threefold degeneracy where M may be dA, dG or dC

1 patient female 73 years old diagnosis gonarthrosis

2 patient male between 65-75 years old

theoretical consideration:

Suggesting that an arbitrary upstream primer detects 3 % of the total RNAs (Liang 1994), then 97 % of the total mRNAs will not be detected, i.e. with 25 arbitrary oligodecamerprimer and the four degenerate T<sub>12</sub>VN primers, about half of the mRNAs would be seen ( $P = 1 - (0,97)^n = 1 - (0,97)^{25} = 53,3 \%$ ) in 100 lanes of high resolution sequencing gels.

Table 2 IL-1 mediated differentially displayed cDNA fragments of human articular chondrocytes

Fragment	bp	IL-1	Features	Stat.sig.score
TAO 8/2(2)	275 bp	+	146 bp sequenced, no homology found	0.999
TAO 16/1(2)	450 bp	+	80 bp sequenced, no homology found	0.69
TAO 16/2(2)	200 bp	+	115 bp sequenced, no homology found	0.04
TAO 17(c)	412 bp	+	412 bp sequenced, no homology found	0.016
TAO 19(c)	209 bp	--	209 bp sequenced, no homology found	0.99
TAU 1/1(1,2)	450 bp	--	100 % sequence identity to human osteopontin cDNA in 303 bp overlap (303 bp seq.)	$1.2 \times 10^{-101}$
TAU 1/2(2)	430 bp	+	188 bp sequenced, no homology found	0.82
TAU 7/1(1,2)	500 bp	+	87 % sequence identity to human cDNA clone c-1sd02 in 125 bp overlap (235 bp seq.)	$8.1 \times 10^{-33}$
TAU7/2(c)	202 bp	+	99.5 % sequence id to human TNF stimulated gene-6 in 202 bp overlap	$4.8 \times 10^{-76}$
TAU 10(1)	400 bp	+	181 bp sequenced, no homology found	0.9997
TAU 12/1(1,2)	470 bp	--	319 bp sequenced, no homology found	$3.3 \times 10^{-14}$
TAU 12/2(1)	390 bp	--	155 bp sequenced, no homology found	0.0078
TAU 12/3(1,2)	250 bp	--	95 % sequence identity to human cDNA clone HRBBA21 similar to S10 in 158 bp overlap (162 bp seq.)	$1.0 \times 10^{-28}$
TAU 13/1(1)	600 bp	+	145 bp sequenced, no homology found	0.12
TAU 13/3(1,2)	500 bp	--	439 bp sequenced, no homology found	0.33
TCO 16/1(c)	241 bp	+	241 bp sequenced, no homology found	$2.4 \times 10^{-7}$
TCO 16/2(c)	230 bp	+	230 bp sequenced, no homology found	$4.3 \times 10^{-5}$
TCO 17(c)	169 bp	+	169 bp sequenced, no homology found	0.49
TCO 18(c)	168 bp	+	168 bp sequenced, no homology found	$1.3 \times 10^{-6}$
TCU 2/1(1)	400 bp	+	178 bp sequenced, no homology found	0.66
TCU 2/2(1)	210 bp	+	151 bp sequenced, no homology found	0.0074
TCU 9/1(2)	430 bp	+	99 % sequence identity to human cDNA clone 131036 3' in 155 bp overlap (155 bp seq.)	$7.2 \times 10^{-88}$
TCU 9/2(2)	320 bp	--	188 bp sequenced, no homology found	0.22
TCU 10(2)	320 bp	--	100 % sequence identity to human cDNA clone 26518 3' in 85 bp overlap (91 bp seq.)	$2.9 \times 10^{-28}$

Fragment	bp	IL-1	Features	Stat.sig.score
TCU 14(1,2)	280 bp	+	99.3 % sequence identity to human cDNA HL60 3'directed Mbol in 249 bp overlap (249 bp seq.)	$3.5 \times 10^{-81}$
TGO 20(1,2)	300 bp	+	304 bp sequenced, no homology found	0.95
TGU 5(c)	317 bp	+	317 bp sequenced, no homology found	0.088
YGU 8(2)	320 bp	+	100 % sequence identity to human 28S rRNA in 58 bp overlap (58 bp seq.)	$1.4 \times 10^{-16}$
TGU 9/1(2)	280 bp	+	169 bp sequenced, no homology found	0.55
TGU 9/2(2)	220 bp	--	100 % sequence identity to human cDNA clone 12A10B in 100 bp overlap (173 bp seq.)	$4.0 \times 10^{-36}$
TGU 12(c)	208 bp	--	87 % sequence identity to human cDNA clone 113442 3' in 208 bp overlap	$5.5 \times 10^{-83}$
TGU 13/1(c)	322 bp	+	322 bp sequenced, no homology found	$6.9 \times 10^{-13}$
TGU 13/2(2)	300 bp	--	94.9 % sequence identity to human F1 ATPase $\beta$ -subunit in 137 bp overlap (137 bp seq.)	$2.3 \times 10^{-43}$
TTO 16/2(c)	239 bp	+	97.5 % sequence identity to human ERCC5 in 239 bp overlap (239 bp seq.)	$9.3 \times 10^{-88}$
TTO 20/1(c)	314 bp	+	100 % sequence identity to human fibronectin in 314bp overlap (314 bp seq.)	$1.9 \times 10^{-121}$
TTO 20/2(2)	400 bp	+	152 bp sequenced, no homology found	0.035
TTU 2/1(2)	300 bp	--	100 % sequence identity to human cDNA clone 118470 5' in 146 bp overlap (146 bp seq.)	$2.1 \times 10^{-36}$
TTU 2/2(c)	184 bp	--	99 % sequence identity to human calnexin in 184 bp overlap (184 bp seq.)	$2.3 \times 10^{-64}$
TTU3(1)	400 bp	+	97 % sequence identity to human NADH-DH mtDNA subunit in 203 bp overlap (203 bp seq.)	$8.6 \times 10^{-69}$
TTU 5/1(2)	300 bp	--	147 bp sequenced, no homology found	0.0065
TTU 5/2(2)	270 bp	--	118 bp sequenced, no homology found	0.035

Fragment	bp	IL-1	Features	Stat.sig.score
TTU 9/1(1)	350 bp	+	94 % sequence identity to human cDNA clone 83764 3' in 159 bp overlap (159 bp seq.)	$5,9 \times 10^{-23}$
TTU 9/2(2)	320 bp	--	149 bp sequenced, no homology found	0,22
TTU 13(1,2)	350 bp	+	194 bp sequenced, no homology found	0,57

Thus, the 44 identified fragments can be subdivided as follows:

1) 2 fragments with sequence homologies to known human genes with known roles in IL-1 mediated processes:

TAU 7/2 identical with human TNF-stimulated gene-6  
TTO 20/1 identical with human fibronectin

2) 6 fragments with sequence homologies to known human genes, whose function in IL-1 mediated processes can be speculated:

TAU 1/1 identical with human osteopontin  
TGU 8 identical with human 28S ribosomal RNA gene  
TGU 13/2 identical with human F1 ATPase  $\beta$ -subunit  
TTO 16/2 identical with human ERCC5  
TTU 2/2 identical with human calnexin  
TTU 3 identical with human NADH-DH mtDNA subunit

3) 9 fragments with sequence homologies to human genes, identified in human genome sequencing projects:

TAU 7/1 identical with human cDNA clone c-1sd02  
TAU 12/3 identical with human cDNA clone HRBBA21  
TCU 9/1 identical with human cDNA clone 131036 3'  
TCU 10 identical with human cDNA clone 26518 3'  
TCU 14 identical with human cDNA clone HL60 3' directed Mbol  
TGU 9/2 identical with human cDNA clone 12A10B  
TGU 12 identical with human cDNA clone 113442 3'  
TTU 2/1 identical with human cDNA clone 118470 5'  
TTU 9/1 identical with human cDNA clone 83764 3'

4) 27 fragments without sequence homologies to known human genes. The detection of TSG-6 and fibronectin, both genes known to be upregulated by IL-1, points to the importance of those other cDNA fragments in the light of IL-1 mediated processes. Those genes very likely play roles in degenerate joint diseases, including rheumatoid and osteoarthritis and with this are interesting candidates as markers for clinical studies or as drug targets for pharmacological intervention.

#### Claims

- Use of osteopontin itself or parts thereof, or antibodies against osteopontin or parts thereof or nucleic acids or parts thereof coding for osteopontin or parts thereof in the diagnosis, prophylaxis or therapy of IL-1 $\beta$  mediated diseases of connective tissues, in particular osteoarthritis.
- Diagnostic aid for the diagnosis of IL-1 $\beta$  mediated diseases of connective tissues, in particular osteoarthritis, containing osteopontin itself or parts thereof, or antibodies against osteopontin or parts thereof or nucleic acids or parts thereof coding for osteopontin or parts thereof.

EP 0 705 842 A2

3. Pharmaceutical for the prophylaxis or therapy of IL-1 $\beta$  mediated diseases of connective tissues, in particular osteoarthritis, containing osteopontin itself or parts thereof, or antibodies against osteopontin or parts thereof or nucleic acids or parts thereof coding for osteopontin or parts thereof.
- 5 4. Use of calnexin itself or parts thereof, or antibodies against calnexin or parts thereof or nucleic acids or parts thereof coding for calnexin or parts thereof in the diagnosis, prophylaxis or therapy of IL-1 $\beta$  mediated diseases of connective tissues, in particular osteoarthritis.
- 10 5. Diagnostic aid for the diagnosis of IL-1 $\beta$  mediated diseases of connective tissues, in particular osteoarthritis, containing calnexin itself or parts thereof, or antibodies against calnexin or parts thereof or nucleic acids or parts thereof coding for calnexin or parts thereof.
- 15 6. Pharmaceutical for the prophylaxis or therapy of IL-1 $\beta$  mediated diseases of connective tissues, in particular osteoarthritis, containing calnexin itself or parts thereof, or antibodies against calnexin or parts thereof or nucleic acids or parts thereof coding for calnexin or parts thereof.
- 20 7. Use of TSG-6 gene product itself or parts thereof, or antibodies against TSG-6 gene product or parts thereof or nucleic acids or parts thereof coding for TSG-6 gene product or parts thereof in the diagnosis, prophylaxis or therapy of IL-1 $\beta$  mediated diseases of connective tissues, in particular osteoarthritis.
8. Diagnostic aid for the diagnosis of IL-1 $\beta$  mediated diseases of connective tissues, in particular osteoarthritis, containing TSG-6 gene product itself or parts thereof, or antibodies against TSG-6 gene product or parts thereof or nucleic acids or parts thereof coding for TSG-6 gene product or parts thereof.
- 25 9. Pharmaceutical for the prophylaxis or therapy of IL-1 $\beta$  mediated diseases of connective tissues, in particular osteoarthritis, containing TSG-6 gene product itself or parts thereof, or antibodies against TSG-6 gene product or parts thereof or nucleic acids or parts thereof coding for TSG-6 gene product or parts thereof.

EP 0 705 842 A2

10. DNA containing a DNA selected from the group consisting of

TA08/2(2)

5           1   CCAAGTTTTT CCAGCAACCC CAAGGGAATA CAGGGAGATC AATGCACCCA  
          51   AAATGGGAAA AGAAAAATAC TTCGATGCAA TGAAACAAAG CCTTTTCCG  
         101   TTCAGTTTCC ATAATTCAGT GGTCACTTTT AAGGCTGCCA CTTGGG

TA016/1(2)

10           1   GACACGAACA CCACATATTT TTATTGGAGG CCCCATGGCT CCTTGAAGC  
          51   CATTTTGGAA CCAAGGGGAC CCACCTTTTT

TA016/2(2)

15           1   CTAAATATAT TCTCTAACAA GTTAATCTCT TTCAAATCTA TAGATAAAAC  
          51   TAAAAGGATA AGGAACCAAG GTTTAACCGA CCTAGCCAAT TATGGCAATC  
         101   ATACTTGCTT TTTAG

TA017(C)

20           1   CATGAAATAT TTCTTGAGGT AATAAGCTTT TACCAAGCTT ATATTTTTGG  
          51   GCAATTCAGT TACAATGAGA AAAAAACACA CCAAAGACC AAAAATTTTA  
25           101   AAACTCACT TTTCTTGCAA TCATAGACAT TTGCATTATT ATAGAACATT  
         151   CAAACAAGTT AGGTGGATAA TTATTGTCTA TAGATAAATA CGATGCAATT  
         201   TTAATAAGAA TTTGAAGAAT GACATTAAAT GCTGTCTGAA GCCTTTGTAT  
         251   TTTTTAATGT ATGACCGATA CTCCGTATAT ACTTAGATAA CTTATCCAGA  
30           301   AACCTCAACT GTATTGAACA TTGCTGAGAG AAATCAACAA TAATTTTAAC

35

40

45

50

55

EP 0 705 842 A2

351 AGATATGATG ACAGNAAAAA TTGATTGCAT ATCTTTTTCG ACTAAAACTT  
401 TTATATTAT TT

TAO19(C)

1 AGAGCAGGGG TATTTTCNCGG TTCATACCGC CATGGCTTAA GAAGCAAAAG  
51 TCATATACCT TAGTAGTGGC AAAGATNGAG GAGATAAAAA AGAGCCTACC  
101 CAAGCTGTTG TTGAAGAACA GGTCTTAGAT AAAGAGGAAC CCTTCCAGAA  
151 GNACAGAGAC AGGCTAAGGG TGATGCTGAG GAAATGGCTC AGAAGAAACA  
201 AGAGATTAA

TAU 1/1(2)

1 CTAAATGCAA AGTGAGAAAT TGTATTTTTT CTCCTTTTAA TTGACCTCAG  
51 AAGATGCACT ATCTAATTCA TGAGAAATAC GAAATTCAG GTGTTTATCT  
101 TCTTCCTTAC TTTTGGGG

TAU 1/1(1)

1 ACATCACCTC ACACATGGAA AGCGAGGAGT TGAATGGTGC ATACAAGGCC  
51 ATCCCCGTTT CCCAGGACCT GAACCCGCCT TCTGATTGGG ACAGCCGTGG  
101 GAAGGACAGT TATGAAACGA GTCAGCTGGA TGACCAGAGT GCTGAAACCC  
151 ACAGCCACAA GCAGTCCAGA TTATATAAGC GGAAA

TAU1/2(2)

1 CCGGAATGGG GAGCRAACTA TAAGAACCGG GACCAGTTTC CTCTCTTTGT  
51 GCCCTAGTTC CCCCTCCTTT GTATACACCC TCCATCCTGA ATAGACTCTG  
101 GTTCTCAGCG TAACACCGAC AACATTCAAT CCTGTAGAGA AACAAATGTT  
151 AGCTCAGAAG GACACAGCCT TTGAATCATC AGAGAGTT

TAU 7/1(2)

1 GTTAAGAATA ACTAAATAAA AGTTTTAATT AATTAGGAA TATAAAAAAC  
51 TATTAACATT TAATTTTATA ACTGTATCTG CCAAGCAACT TTAAATATAA  
101 TTTATTTACC

TAU 7/1(1)

1 CACGCAATGT GAAATAGGCA CATAGGAAGA ATGGGGAAAC CATCCCCTCA  
51 AGCATTTATC CTTTGAGTTA CAAGCAATCC AATTACACTC TTTTAGTTAT  
101 TTTTAAATGT ACAGTTAGGT TATTA

TAU 7/2(C)

1 CCTTGAAGAT GACCCAGGTT NCTTGGCTGA TTATGTTGAA ATATATGACA  
51 GTTACGATGA TGTCCATGGC TTTGTGGGAA GATACTGTGG AGATGAGCTT  
101 CCAGATGACA TCATCAGTAC AGGAAATGTC ATGACCTTGA AGTTTCTAAG  
151 TGATGCTTCA GTGACAGCTG GAGGTTTCCA AATCAAATAT GTTGCAATGG  
201 AT



EP 0 705 842 A2

TAU10(1)

5           1   GGAGATGACA TTTGCTTTGG GCAGAGGCAG CTAGCCAGGA CACATTTCCA  
          51   CTATAATTTT ACAAAGTTAA ATTTATAAGC TAGCATTAAAG TAAAGTGAAG  
         101   TTCCAGCTCC CTTGCTAAAA ATAAC TAGAG GTAATAATTG GTATTCAGGT  
         151   AACTCATTTA CATCATAATG TGTGTGAAA A

TAU12/1(2)

10           1   TATAAAATAT AAATTATATT ATAAATCATG TATTATTTAT AAAATTATAT  
          51   TATAAATTTA TAAAAATATA AATTATATTT TAGGCTTAAT GTATAAGGAA  
         101   TATAAATTAT TAATAAGCAT ATGA

TAU 12/1(1)

15           1   TGTAATTAAC TGTNCTTGTA GGTCTGTCTT TTATACATGT GTGAGTTTTT  
          51   CTTTACAATA GATTCCTAGC ATTGGGATTG CTAGGTCAGA TGGTATGCAC  
20           101   ATTTGACATT TTGATTGATA GCACCAGATT GCTTTGTTAA AAAATTTTNN  
         151   TTTATAGTTT ACATTATCTT TGTACAATAG ATGTTCTCTT TCGAC

TAU 12/2(1)

25           1   GGGAAGTGAA TTGAAAATAC TTCTTTNTCA ACATAATTTT NGGGTTTTGA  
          51   AATTGTGTTT GGGTTTTCAG GAAATTGGTG GTAATCTTGT ATTAGCTGAA  
         101   AAAAAGTGAA TTTTAAATTT CTCAGTGAAG AAGCRAATGA TTTATTTTTC  
         151   ATAGA

TAU12/3(2)

30           1   TGTTCTGGTA ACTGTTCTAA TTGTGTCTTT GTTACTTCCA GTGCAACCCCT  
          51   TTCAGGTAAG

TAU12/3(1)

35           1   CTAAAGAACT TGGTATCTCT ATTAAGCAC ACGAACCTCC AAGGAAAATA  
          51   GAGCGATTTA CTCTTCTCAT ATCAGTGCAAT ATTTATAAGA AGCACGGAGT  
40           101   CA

TAU13/1(1)

45           1   AGTCATCAAT TCCTTTTTAT CTGTAATTAC ACATTTGTTT TTATTTCAAA  
          51   GTAATTATAA GGTGTTATAT TGCATATAAT CAGAAACTA AATGGAAATA  
         101   AAATTTTAGT AAGCCCGGCC CCTTTGACCG ATACAGAAAA CTTGA

TAU 13/3(2)

50           1   TATATGGCAG TCTAAAGCAT CAAAGATTG CATCAACATC TTTCAATTTA  
          51   GACATCTCCT TGCAATGTAA AATATCATGT ATCAACAACA TCTGGTGCAA  
         101   ATCCATGAGT CTAACGAC ATTCACTTA GCTCGATTAT TATTCCTTCG  
         151   TACAGTCGAT GTAAACAATA CAGAAAGAGG ATTATTAAGA ACAGTTT

EP 0 705 842 A2

TAU 13/3(1)

1 ATTCATGAAA TGGTCTATAT GCATGATATT GTAAATTCGG ACTCGAAACC  
51 GAAACCAAGG ATTCCGTTAC AAAAATTCCT TAATGCTGAG AATGTTCTCA  
101 CGCAAACAAC ATCATGGACA TTAAATTCAA GATATGTGAA TGTTAATTCT  
151 GTCAATAAAG TCAACGTAAA GAGTAAAGTT AAAACAGTT ATATCTNNNC  
201 TGTCAATGAT GAGTTTAGTT TAACAGATGA TGAATCAATT CT

TCO 16/1(C)

1 CAAAGTGTTT TTGGTTTGA GAGAGAGAGA GATTGAGAGA CAGAGAGAGA  
51 GAGAGAAACC AAGGGATCAT GATAGTTATA GTCAAATACG AGGTTGGATT  
101 ATCTTTTGAA AATGTGTTGG TTCTGTGATA CAAGAGGAAG CTAAGACATA  
151 TCGTGGAAC ATCTCCCCC TCCACCTTAA TATCAAGAAC AAATTGTGGA  
201 ATCTAATGTT AATGAGAAGT AGTCCCCAC TGTGTCAGAT G

TCO16/2(C)

1 NCATCTGACA CAGTGGGGAA CTACTTCTCA TTAACATTAG ATTCCACAAT  
51 TTNNNCTTGA TATTAAGGNN NNNNNGGGAG ATCGTTTCAC GATATCGTCT  
101 TAGCTTCCTC TTGTATCACA GAACCAACAC ATTTCAAAG ATAATCCTTC  
151 CTCNNTTGA CTATACTAT CATGATCCCT TGGTTCTCTC TCTCTCTCTG  
201 CTCTCTCATC TCTCTCTCTC TNAACNAA

TCO17(C)

1 ACAGTAGTTA GGAGTTTCTT TACTTACAAA ATCACTGGAA ATGATTAAAT  
51 TGCTTTTCCC CCTCCCCAGA GGTGCATTTT TCTTATTTCC ATATAGTAAA  
101 GTTGAGCTTT TACAGTGCAT AATGTGACAT TTGGAATGCT TATCAACTGC  
151 ATGTAAACAT TAATAACCT

TCO18(C)

1 GTAAATGGTA TTANNGCTG AAGAAAAAAA ATTTTCAAG ACCTCTGTTT  
51 TTTAACTGAA CTTTATCATT GGCATTGTGG GCTTTGAAGT TGCTGGGATA  
101 AATTAATATA ATTAATAAAA AGACTGAATT TAATTGCAA AAAAAAAAAA  
151 AACAATAAGT GTGGTGAT

TCU2/1(1)

1 AAGAAATTAT CCAGTTATTT ACAAGGCCAC TGATATTTTA AACGTCCAAA  
51 AGTTTGTTTA AATGGGCTGT TACCGCTGAG AATGATGAGG ATGAGAATGA  
101 TGGTTGAAGG TTACATTTTA GGAAATGAAG AACTTAGAA AATTAATATA  
151 AAGACAGTGA TAAATACAAA GAAGATT

TCU2/2(1)

1 CGGGTTAATA TTATCCTCTA GTATAAGTGA ATTACTAGTT TCTCTTTATT  
51 TAGACAAACA CACACACACC AGATAATATA AACTTAATAA ATTATCTGTT  
101 AATGTAGATT TTATTTAAAA AACTATATTT GAACATTGGT CTTTCTTGGA  
151 C

EP 0 705 842 A2

TCU9/1(2)

5  
1 ACATAACAGC TTTTATACAA TGATAAGGAC ATATCATTTG TTTACAAAGA  
51 AAGTCTAAAA TTTCAAGAAC ATTCAAAGAG CTAACACAGT AAAGGTCATG  
101 CAAGTTCTAG AATAGTGAAT CATGACAGAA CTCATTCAAT TTATCCTTTA  
151 TCTCC

TCU9/2(2)

10  
1 AAGTATGGGT AGCTAAATTT GCATTAAATT AAAAGTACAT ATAATGCAAC  
51 ACCACTCTAC ATCTGTATAC CTACGAATGT ATGTGTACTA CACACCCTTA  
101 AAATGTTTTT CAAAGTCTTA ATATATTAGA ACATGTTTTT ATTTTTTCAT  
15 GGGATGTTAA TACTATTCTA TGATTAAGAA AATACTAG

TCU10(2)

1 AATACAGTTA TTCTAGCTTT TCATATTCAA TTTGAATGAT CAGAAAAGTA  
20 51 TATTAGTCAC ACAGAATTAA ATATTTTAGA TAGTAAGAAT C

TCU14(1)

1 ATCCTTAGTA AGTGGATTTT GGGGAAAAAA GCACCTGGGC TTCTGGTTCT  
25 51 TTTTGATAAT ATATAAAATT ATTCATTATG AGGTTGCAGT TGTTTGCAAA

TCU14(2)

1 GAAGTGAAAG TCAGCCCTTT AGCTATTATT TATTGCTTTA TTAGAGCAGA  
51 GGGAAGTGAC ACTCATGGCC TTCACAGAGC TCTGCAGAAA TATATGCACA  
30 101 GAGTGGTCAA TGCCAACATC TGAGTAAGTC TTCCAAA

TGO20(2)

1 CAGAACATTA GGATTTATTC CTTGATTAGT TCAAATGATT TCAACAGCTG  
35 51 AATTCCTTGA GATGTGTAAG GCAGGTTGGT CCTTTGGATG GACTGTAGAC  
101 TGAAACTTCC TATAACTGTA GTGATATGTA CACAGCTACA TAGCAAAGTG  
151 CTTTATTATG AAAATGAAGA A

TGO20(1)

40 1 CAGTGTGAGA GTCTCATTTT TATGCACAGT GTTTCTCAGG AGGATGGAGC  
51 TAGTTAGCTG TCTGTTGTCT GTAGCCCAGC TTGATAATGG AACTATACAG  
101 CGAAGAGACA ATCTCTGGCA AGTTTTTGTG GAA

TGU5(C)

45 1 TTAGAGTAAA ATTCCAAATA AATGCTTTGC TCCAAAATTA CACTAACCAG  
51 GCTGGGTCTC TATCATACAT CTTCAATACC CTCAAACCTA GATTGTAAAG  
101 TGAAAAAAGT GATTAGCNNT TCCATTTGTT CATCTGTCA CTCACATTCT  
50 151 TAGGCATTTT AAGGATGAGC AACCTTTGTT TCAGAAAGGG TAAGTAATTA  
201 GCCCCCTGGA GGTTACATAG TTATAATTA GTCTTCAGAA TCCGTTTCGAA  
251 GGGNNNNGTT ACTATTTTAA AGATAATTAG AACCCACCTT GTAGCAATAA  
301 AAGTTTTCTT GTCTTTG

55

EP 0 705 842 A2

TGU8(2)

5 1 GCGAAAGACT AATCGAACCA TCTAGTAGCT GGTTCCTCC GAAGTTTCCC  
51 TCAGGATA

TGU9/1(2)

10 1 TTAATGTTTA AATACTACTT TTTTTC AAG CTTGCCCTAG ATACCAACTG  
51 TTTATCTAAC ACACAATTCC AGTGTGCGCA AGCCTCATGC CAATTTGAAG  
101 GGAACAGCCA AAACCTATGC ATTCATATAA AAAGAGTCTC TAGGCTCTTA  
151 TATCTACATT ATAATTTT

TGU9/2(2)

15 1 GGAATAACAT TTTTATGA GGAACCCCTT TAAATGGAT GCACACAGTG  
51 GCATTTTCTC CTAGGCTCAA AGCTGAGTAC ACTCCGTAA TTTAATAAT  
101 ATTTAGGCA AGTCTATGA CAATTATACC AACAGTTTC TTCAACCCCA  
20 151 CCACCACCCC ACCATCTCTA TGC

TGU12(C)

25 1 GGAGGAAGCT TTATTGGGA AGAGTCCGGT TCNNTCGGCC CTGATCAGCT  
51 CTAGCCTGCC CACCCCATCT CAGCCAGGCG GCTTACTTC TTCCTGAGCT  
101 TCAGGTCTTT CTTCTCCTG ATTTCTTGG CCAGCTCCCC AATCAATCTC  
151 CAGTACTCAT TGAACCTGAG CTCCGAGNCC TGATTACAT CCAAGCTCTT  
201 CATCTTCT

TGU13/1(C)

30 1 GGATGTGGTA GTTGATCTTT AATGCCCAT CTAGGTCGGA AAAATCCATG  
51 ATCCTAATT TTAAGAGAAG GTTGGTAACT CTACTTAGGA CTTTTTTTG  
35 101 TAAGAGGAAT AATGTAGCCT CACCCTATC TTTCTGAAA TGTTTAAACC  
151 ACTGAAATAT GGAGATCAA TCCAGCTTAC AACTGGTAA CTCAAATACT  
201 ATTTTTTTT TAACTATCT TTTCTAACT AATCACCCT CTTGTACATA  
251 GAACTTTCTA TCTCAGTGCC AATTCTTAGA GGTGATGCA AACAGCTCTC  
40 301 CAGAGGCCT GTGCTATTGT TC

TGU13/2(2)

45 1 GGGGTGTACA TTTATTGGA AACCTTAAAT ACTGTTGAGA AAGAATATAT  
51 CTTCAATCAA GGTCTTGCCG AGCCTACACA GAAAAATGAA GCTTTTGGG  
101 TTAGGGGCAA GGATATATAC AGTACAGAGG ACAAAGA

TTO16/2(C)

50 1 ACATTCATTA AAGATGAACT TTCAGCATCT TCACTTGAAG ATCCATCAGA  
51 TGATTCTGAG AGGCAGGTCT CCCCCAAAAA TCCACCGCAT GTATTCTTTC  
101 GTTTAGAATC TGAAAGCCTC TTTCTTTTCA GGCTTGATGA CTCTCTAAG  
151 GTATTGTGA TGCTCTCTT CTGGGTTTTT CGTTTTGCCT TATCAAGTAG  
55 201 CTNAAATTCA AACACCATGG CAANAGAAAC TGCTTCTAT

EP 0 705 842 A2

TTO20/1(C)

5 1 CCACCAGCCT ACTGATCAGC TGGGATGCTC CTGCTGTCAC AGTGAGATAT  
51 TACAGGATCA CTTACGGAGA AACAGGAGGA AATAGCCCTG TCCAGGAGTT  
101 CACTGTGCCT GGGAGCAAGT CTACAGCTAC CATCAGCGGC CTAAACCTG  
151 GAGTTGATTA TACCATCACT GTGTATGCTG TCACTGGCCG TGGAGACAGC  
10 201 CCCGCAAGCA GCAAGCCAAT TTCCATTAAT TACCGAACAG AAATTGACAA  
251 ACCATCCCAG ATGCAAGTGA CCGATGTTCA AGACAACTGT TTTAATAAAA  
301 GATTACATT CCAC

TTO20/2(2)

15 1 TTGGTACCAC AGTCACAGAA CTGGGGGTCA TTTTCTAGAT GAAACAAACG  
51 GAACAAGTTC TCTTCCAACA AAGAAATGTA CTGTAGAAAT TAATTTCTCT  
101 CATGAATTTT ATATATTGTG TACAAATATA AGGTATGTAT CTGAATACAA  
151 AG  
20

TTU2/1(2)

1 CTAGAACTTC CAAAGGCTGC TTGTCATAGA AGCCATTGCA TCTATAAAGC  
51 AACGGCTCCT GTTAAATGGT ATCTCCTTTC TGAGGCTCCT ACTAAAAGTC  
25 101 ATTTGTTACC TAAACCTTAT GTGCCTTAAC AGGCCAATGC TTCTCG

TTU 2/2(C)

30 1 AACCAGTATT TCAAACTAT TATCTGGATT CAAGATTAGT GTGTAAAGAT  
51 TGTTTTCTTA TCAGTAAAT AGGTCTTCAG ATCTGCATCT GGCCTCTTAG  
101 CATGTTTTTC TTCATAGATA CCCGTTTTGG GGTTTTTGCG TCGGAAGATG  
151 AAGTGCAGTT TATAGTCCTC TCCACATTTA TCTG

TTU3(1)

35 1 GGGTAGAAAG CTGAATAATT TATGAAGGAG AGGGGTCAGG GTTGATTCCG  
51 GAGGACCTAT TGGTGCGGGG GCTTTGTATG ATTATGGGCG TTGATTAGTA  
101 GTAGTTACTG GTTGAACATT GTTTGTTGGT GTATATATTG TAATTGAGAT  
40 151 TGCTCGGGGG AATAGGTTAT GTGATTAGGA GTAGGGTTAG GATGAGTGGG  
201 AAG

TTU 5/1(2)

45 1 GACAAAAAAA AAAAAACAGG TTTTAAAGCT AGAAATGAAA AGCTACTTAA  
51 GTATCTTAAA GGATAAGTTA CTTTATTATA CACTAGAAAC ATACACAATA  
101 GCTGAAAACT TAAAAAATCT CACACTGCTG AATGTCTCTG CTGGCTG

TTU5/2(2)

50 1 GCATCCATTG TACATTGTTT GGTITGAGGT TACCATGAGG CCTGTAAATA  
51 CTATCTTATA ATTTATTATT TCAACCTGAT AAAACTTAAC ACTATTTGCA  
101 TAAACAAACA AACGAAAA

55

TTU9/1(1)

5  
1 TAAAATACTG GTTCTTTTAT TCTGCAATAT TTTTAAAAAT CACATTTTCA  
51 GCCAGGCGCA GTTTCCACACA CCTGTAATCC GGCACCTTGG GAGGCTGAGA  
101 TGGGTGGATC ACAAGGTAGG AGATCAAACA TCCTGGCCAA CATGGTGAAC  
151 CTGTTTACT

TTU9/2(2)

10  
1 CAAGTATGGG TAGCTAAATT TGCATTAAAT TAAAAGTACA TATAATGCAA  
51 CACCACTCTA CATCTGTATA CCTACGAATG TATGTGTACT ACACACCCTT  
15 101 AAATGTTTCA AAGCTTAATA TATTAGAACA TGTTTTTCATT TTCAGGGAG

TTU13(2)

20  
1 GGAAATACAC TAGCATGTGA GCACTGTATA TAAAGCTTGA GGTTAGGAGG  
51 TAAAAAGAAA GAAATCATT TTAACCTCCTA AGATGT

TTU13(1)

25  
1 TGAATTAAAT GGAATCGTTG AAAGGACAAG GAGATCGGTA ATATCTCTCT  
51 AAAGAACTTA TATACTAAAA TCTGTAATTG CCTGTACCAA AAGTTTTAGT  
101 CTTCTTTT

or an analog thereof.

30 11. Vector containing a DNA according to claim 10.

12. Host cell containing a vector according to claim 11.

35 13. Method for isolating a gene inducible by treating chondrocytes with IL-1 $\beta$  containing the steps:

- (a) hybridizing a DNA according to claim 10 under stringent conditions against DNA or RNA containing said gene; and  
(b) isolating said gene.

40 14. A method according to claim 13 wherein said DNA or RNA has been isolated from chondrocytes, particularly human chondrocytes, that were treated with IL-1 $\beta$ .

15. Process for expressing a gene isolated according to claims 13 or 14 containing the steps:

- (a) cloning said gene into a suitable expression vector; and  
(b) expressing said gene in a suitable host cell.

50 16. Method for producing a protein containing the steps:

- (a) culturing a suitable host cell containing a vector which contains a DNA according to claim 10 or a gene produced by a method according to claim 13 or 14; and  
(b) isolating the expressed protein.

55 17. Diagnostic aid containing a DNA according to claim 10 or parts thereof or a gene isolated according to claim 13 or 14 or parts thereof.

**EP 0 705 842 A2**

**18.** Use of a DNA according to claim 10 or parts thereof or a gene isolated according to claim 13 or 14 or parts thereof for the diagnosis, prophylaxis or therapy of IL-1 $\beta$  mediated diseases of connective tissues, in particular osteoarthritis or rheumatoid arthritis.

**5 19.** Use of a gene isolated according to claim 13 to 14 for the production of a pharmaceutical.

**10**

**15**

**20**

**25**

**30**

**35**

**40**

**45**

**50**

**55**